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PCT

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(54) Title: LEPTIN ASSAY

(57) Abstract

A trans-activation assay for leptin is described. Leptin response elements are located proximal to a promoter, and the promoter region is operatively linked to a reporter gene. When leptin binds with the receptor, the reporter gene is transcribed.

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TITLE OF THE INVENTION
LEPTIN ASSAY

FIELD OF THE INVENTION

- 5 This invention relates to an assay for determining the presence of compounds which bind to a leptin receptor. This invention also relates to an assay for quantifying the amount of a leptin receptor binding agent present in a sample.

10 BACKGROUND OF THE INVENTION

- Leptin is a product of the Ob gene (Zhang *et al.*, 1995 *Nature* 372:425-432). In its absence, mice develop extreme obesity resembling morbid obesity of humans. They also develop diabetes resembling human type II diabetes (Zhang *et al.*, 1995 *Nature* 372:425-432), and become infertile (Chehab *et al.*, 1996 *Nature Genetics* 12:318-320). Repletion of leptin reverses these pathologies.

- Currently, the only manner in which preparations of recombinant leptin can be assayed is by injection of large quantities into *ob/ob* mice (Campfield 1995 *Science* 269:546-549; Rentsch 1995 *Biochem Biophys Res Comm* 214:131-136). Leptin activity is assessed based upon depression of feed intake and is highly variable among individuals. No mutually agreed upon standard of leptin activity exists. Therefore, a rapid, accurate assay for leptin bioactivity which utilizes small amounts of leptin would be desirable.

25 DETAILED DESCRIPTION OF THE INVENTION

- This invention relates to a method for determining if a leptin receptor binding compound is present in a sample comprising: contacting the sample with a cell which comprises a) nucleic acids comprising a promoter region, said promoter region comprising a promoter and at least one leptin response element; said promoter region operatively linked to a reporter gene; and b) nucleic acids encoding a leptin receptor; and
- 30 determining if transcription of the reporter gene occurs.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing functional assay of leptin using GT1-7 cells. Cells were either: mock transfected with respect to ob-r, transfected with lean rat Ob-r cDNA, or transfected human Ob-r cDNA and showed induction of luciferase activity in cell expressing Ob-r treated with leptin.

Figure 2 is a graph showing that cells respond in a linear, dose-responsive manner to the addition of leptin as determined by increase in luciferase activity. GT1-7 cells were transiently transfected with human Ob-r and treated with various amounts of human leptin.

Figure 3 is the DNA sequence for lean rat Ob-r.

Figure 4 is the DNA sequence for human Ob-r which has been placed in the pCMV4 vector. The Gen Bank accession number for human ob-r is U43168. Nucleotides 141-37-7-0 are used in pCMV4-Ob-4.

Figure 5 is the DNA sequence for *fatty* rat Ob-r.

As used throughout the specification and claims, the following definition shall apply:

"Leptin receptor binding compound" means a compound which binds to a leptin receptor.

"Leptin mimetic" means a compound other than leptin which binds to a leptin receptor and triggers a cascade of intracellular reactions which ultimately results in transcription of DNA under the control of leptin response elements.

"Leptin response elements" means DNA sequences located within a promoter region which are responsive to a leptin receptor binding event, and in the presence of such an event, allow for transcription of the DNA under the control of the promoter.

"Promoter region" means DNA located upstream of a protein or peptide encoding sequence, and includes at least one response element.

"Promoter" includes full length promoters, minimal promoters and promoters which are less than full length, but include more nucleic acids than minimal promoters.

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"Ob-r" means Ob-receptor.

One aspect of this invention is a cellular "trans" activation assay which can determine if a particular compound can bind to a leptin receptor. In general, the binding of a ligand to its membrane-bound
5 receptor initiates an intercellular cascade of signals. Ultimately, a specific transcriptional control element is activated, leading to the transcription of DNA. It has been found, in accordance with this invention, that the binding of native leptin with its native leptin receptor
10 initiates a cascade which activates leptin response elements, leading to the transcription of DNA. One such leptin response element which has been identified in accordance with this invention is an IRF-1 derived gamma-interferon activation sequence. IRF-1 derived activation
15 sequences have been described in the art (Pine *et al.*, 1994 *EMBO J.* 13:158-167) but their responsiveness to leptin-leptin receptor binding events has been heretofore unknown.

This discovery of a leptin response element has allowed for the design of various assays which form the basis of this invention. A promoter region is constructed which contains a promoter of choice and one or more leptin response elements. The promoter region may
20 contain be a hybrid promoter region, i.e. contain a promoter and response elements which do not naturally occur together, or they may be a naturally occurring promoter region. In a preferred embodiment the promoter is a well-characterized promoter, such as the herpes
25 simplex virus thymidine kinase promoter, but any promoter which is known to function in the host cell chosen may be used. It is also preferred that the promoter be a minimal promoter, so that there are no transcriptional control sequences which may influence the activity of the leptin response element(s).

The leptin-response element is preferably placed proximal
30 to the promoter region. Intervening sequences may be present between the leptin response element and the promoter, provided they do not interfere with the functioning of the leptin response element.

In accordance with this invention, a suitable leptin response element is an IRF-1 derived gamma interferon activation sequence

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5'-CTGATTTCCC CGAAATGACG-3'. In a preferred embodiment, the promoter region comprises a plurality of IRF-1 derived gamma interferon activation sequences; and more preferably it comprises at least three such sequences. When a plurality of leptin response elements
5 are present, they are preferably joined in tandem. However, there may be intervening sequences present provided they do not interfere with the functioning of the leptin response elements.

In order to detect transcription which occurs as a result of the leptin-leptin receptor binding, it is preferred to operatively link a
10 reporter gene to the promoter region which contains at least one leptin response element. A reporter gene may be any gene which encodes a peptide which is easily detected, or otherwise allows for easy detection of transcription or translation. It generally encodes a protein which
15 does naturally occur in the host cell or only is produced in small amounts by the host cell. Examples of well known reporter genes include: chloramphenicol acetyl transferase (CAT), green fluorescent protein (GFP), luciferase (either bacterial or firefly), and other
enzyme-based detection systems such as β -galactosidase, alkaline phosphatase, and the like. In a particularly preferred embodiment,
20 luciferase is a reporter gene. In alternative embodiments, the mRNA transcribed from the reporter gene DNA may be measured rather than the translation product.

The reporter gene construct, which comprises a) the promoter region which contains a promoter and at least one leptin
25 response element and b) the reporter gene operatively linked to the promoter region forms yet another aspect of this invention. It is preferably placed in an appropriate vector and is used to transfect a host cell. This vector comprises yet another aspect of this invention. The vector may be any known vector, including plasmids, cosmids and viral
30 vectors which can function in a chosen host cell.

The host cell may be any cell or cell line which is conveniently cultured. In general, a preferred host cell will be eukaryotic, preferably mammalian, and in particularly preferred embodiments it is a mouse hypothalamic cell (such as GT1-7), a

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mammalian neuroblastoma cell line, mouse fibroblast cell lines such as NIH/3T3 (ATCC CRL 1658) or L (ATCC CCL 1), Chinese hamster ovary CHO-K1 cells (ATCC CCL 61) or human embryonic kidney-derived 293 cells (ATCC CRL 1573).

5 The host cell should either have an abundance of leptin receptors on its surface or it can be transformed to express an abundance of leptin receptors on its surface, the latter situation being the preferred one. Any leptin receptor may be used; human and rodent ones are preferred. Nucleic acids encoding human leptin receptors have
10 been described (Tartaglia *et al.*, 1995 *Cell* 83:1263-1271 which is hereby incorporated by reference) and are shown in Figure 4. Mouse genes, can also be used, especially the position 26 to 2775, based on the sequence given in Gen Bank. Nucleic acids encoding leptin receptors from wild-type rats, *fatty* rats (*falfa* genotype) (shown in Figure 3) and
15 rats heterozygous for the *ob* receptor gene (shown in Figure 5) are described in co-pending U.S. application Serial Nos. ____ and 60/013,969, Attorney Docket Nos. 19462PV filed February 22, 1996 and 19462PV2, filed March 22, 1996, both of which are hereby incorporated by reference. By transfecting cells with various leptin
20 receptors, the biological response of receptors from different sources can be studied and compared. Also, leptin receptors containing particular desired mutations can be constructed according to known molecular biological techniques and the structure and function of the mutants can be studies using the assay of this invention.

25 Another aspect of this invention is a set of vectors suitable for transfecting a host cell so that it can be used in the assays of this invention. The set of vectors comprises a first vector which contains a leptin receptor construct. The leptin receptor construct includes nucleic acids encoding a desired leptin receptor or mutated form of the
30 receptor. It may be under the control of its native promoter or any other desired heterologous promoter. Optionally it may also contain other expression-control elements, such as enhancers and sequences which assist in expressing the receptor on the membrane.

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The set of vectors also comprises a second vector comprising the receptor gene construct described previously.

In a preferred assay of this invention, compounds which are suspected of being leptin receptor ligands can be assayed. In this embodiment, cells which express a leptin receptor (either natural or recombinant) are transfected with the reporter gene construct described previously and is transfected with a leptin receptor construct, if the cell does not naturally express an abundance of the leptin receptor. The putative leptin receptor ligand is placed in contact with the transfected cells, and the presence of the reporter gene transcription or translation product is detected. This may be compared to the amount of transcription or translation measured in a control assay, where an identically transfected cell is placed in contact with leptin. A further advantage of the assay of this invention is that it is dose-responsive; i.e. as more leptin ligand-leptin receptor binding occurs, transcription and/or translation of reporter gene increases, and therefore allows for a quantitative determination of leptin receptor binding activity.

A counterscreen may also be employed as a part of this assay. In the counterscreen, a second cell of the same cell type is transfected with the reporter gene construct described previously, but is not transfected with the leptin receptor gene construct. The putative leptin receptor ligand is placed in contact with the transfected cells and the presence of the reporter gene transcription or translation is detected. Those putative leptin receptor ligands which activate the reporter gene construct only in the presence of leptin receptor are determined to be specific leptin agonists.

Using this embodiment of the assay, leptin agonists and antagonists may be identified. A leptin agonist is a compound which binds to the leptin receptor, such as a leptin mimetic, and produces a cellular response which is at least about equivalent to that of leptin, and which may be greater than that of leptin. Such compounds would be useful in situations where leptin insufficiency causes obesity, diabetes or infertility.

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Also using this embodiment of the assay, leptin antagonists may be identified. A leptin antagonist is a compound which can bind to the leptin receptor, but produces a lesser response than that of native leptin. Such compounds would be useful in the treatment of anorexia and cachexia.

In another aspect of this invention, novel leptin response elements can be identified by inserting various putative leptin response elements proximal to a promoter which controls the transcription of a reporter gene. A cell transfected with this construct and expressing natural or recombinant leptin receptors is contacted with leptin. The resulting transcription of the reporter gene is compared with that which occurs when the leptin response element is an IRF-1 derived gamma interferon activation sequence. Novel leptin response elements which are identified using this assay form yet another aspect of this invention.

In another embodiment of this invention, the potency of a leptin preparation can be determined, and quantified. In this embodiment, the leptin-containing preparation is contacted with the cell containing the reporter gene construct and which is expressing leptin receptors (either recombinant or native). The amount of transcription and/or translation of the reporter gene is measured and is compared to that obtained using preparations of known potency.

In yet another aspect of this invention, the biological activity of various leptin receptors can be studied and compared. In this embodiment, a first cell is transfected with a reporter gene construct and a first leptin receptor. The first leptin receptor can be any leptin receptor or variant or mutant whose activity is to be determined. The first cell is put into contact with leptin and the reporter gene transcription or translation is measured. This amount is compared to that obtained under the same assay conditions using a second leptin receptor whose activity is known or is otherwise to be used as a reference.

The following non-limiting Examples are presented to better illustrate the invention.

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EXAMPLE 1Vector Construction

AH32, an expression vector transactivated by leptin, was constructed as follows. Two complimentary oligonucleotides containing the Stat Binding Element (SBE) from the IRF-1 gene (Sims *et al.*, 1993 *Mol Cell Biol.* 13:690-702; and Pine *et al.*, 1994 *EMBO J.* 13:158-167; both of which are hereby incorporated by reference) were synthesized, kinased and annealed using standard molecular biology techniques (Sambrook *et al.*, 1989 *Molecular Cloning: A Laboratory Manual*, 2nd Ed, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). The sequences of the oligonucleotides used is 5'-CTGATTTCCC CGAATGACG-3' and 5'-CGTCATTTCG GGGAAATCAT-3'. The double stranded oligonucleotides were cloned into a *Sma I* site of pTKLuc. Plasmid pTKLuc contains a promoter sequence from -35 to +10 of the herpes simplex virus thymidine kinase gene (McKnight *et al.*, 1982 *Science*, 217:316-324) upstream of the firefly luciferase gene found in pZLuc (Schadlow, *et al.*, 1992 *Mol. Biol Cell* 3:941-951). The ligated DNA was used to transform *E. coli* using DH5 α competent cells (Gibco/BRL, Gaithersburg, MD). DNA obtained from the transformed colonies was analyzed by restriction digest analysis and the orientation and number of copies of SBE oligonucleotides found in each of the transformants was confirmed by sequencing through the insert using a sequencing kit (US Biochemical, Cleveland, OH). Clone AH32 was found to contain 3 copies of the SBE inserted in the same orientation and was used for further studies with a human fibroblast cell line (WI-38 VA13 subline 2RA; ATCC No. CCL 75.1). Cells transfected with the AH32 showed a 6-10 fold increase in the luciferase activity after 6h of treatment with IFN- α or IFN- γ .

Primers ROBR 35 (5'-TTGGAGGACT ATGGGTGTC-3') and ROBR 37 (5'-CTACTGGAAT GGAACCTGG-3') were used to obtain the full length coding sequence for the rat OB-R, (approximately 3.7 kb in size) by the PCR using rat hypothalamic cDNA as the template. Long PCR produced the full length fragments by a modified method of Barnes (Barnes, 1994, *Proc. Natl. Acad. Sci.* 91:2216-2220,

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which is hereby incorporated by reference). For the long PCR reactions, Taq Extender (Stratagene, La Jolla, CA) and the Expand Long Template PCR System (Boehringer Mannheim, Indianapolis, IN) were used in combination. The standard PCR reaction mix, in a final
5 volume of 20 μ l, consisted of 40 ng of template (rat cDNA), 200 ng of primers, 500 mM dNTPs, 1 X Buffer 3 from the Expand kit, and 0.2 ml of the Expand kit enzyme mix. Reactants were assembled in thin walled reaction tubes. The amplification protocol was 1 cycle of 92°C for 30 sec., followed by 35 cycles at 92°C for 30 sec., 54°C for 1 min. and
10 68°C for 5 min. using a Perkin Elmer 480 Thermal Cycler.

The DNA sequence of the full length products was determined using ABI PRISM Dye terminator cycle sequencing with AmpliTaq DNA polymerase, FS (Perkin Elmer, Foster City), and a number of PCR induced errors were detected. To eliminate these PCR
15 induced mutations, restriction fragments from clones containing the correct DNA sequence were assembled. Three different restriction fragments were used to construct a full length clone derived from *falfa* hypothalamic cDNA, with a C at nucleotide 880. The expression construct was obtained by ligating a fragment from a hind III polylinker
20 site to a *Nde* I site at nucleotide 502, a fragment from the *Nde* I site at 502 to a *Sac* I site at 1522, and a fragment from the *Sac* I site at 1522 to a *Xba* I polylinker site into the *Hind* III and *Xba* I sites of pcDNA 3 (Invitrogen, San Diego, CA). The wild type (lean) clone, with an A at nucleotide 880, was constructed by replacing the *Nde* I by *Sac* I
25 fragment from 502 to 1522 with a fragment derived from lean hypothalamic cDNA.

pCH110, a vector expressing β -galactosidase under control of the SV40 early promoter (Hall *et al.*, 1983, *J Mol Appl Gen* 2:101-109), was obtained from Pharmacia Biotech, Inc. (Piscataway, NJ).

30 A fragment of the human Ob receptor cDNA, nucleotides 141-3770, containing the entire coding region was obtained using PCR and subcloned into pCMV4 (Andersson, S. 1989 *J Biol. Chem.* 264:8222-8229) a gift of D.W. Russell.

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EXAMPLE 2

Cell Culture

GT1-7 cells, a mouse hypothalamic cell line, a gift of P. L. Mellon (Liposits *et al.*, 1991 *Endocrinology* 129:1575-1583) were maintained in Dulbecco-modified Eagle Medium (DMEM) (Gibco/BRL, Gaithersburg, MD), 10 % bovine calf serum, 50 µg/ml streptomycin, and 50 U/ml penicillin (growth medium) at 37°C in an atmosphere of 95% air, 5% CO₂. Cells were seeded at approximately 4x 10⁴/cm² and passed before confluence.

EXAMPLE 3

Transfection of GT1-7 Cells

18 hours before transfection, cells were plated at 3.5 x 10⁵ cells per 35 mm tissue culture dish. The plasmids AH32, appropriate Ob-r plasmid and pCH110 were introduced into cells using liposome-mediated transfection. Specifically, 300 ng of each plasmid was added to 200 µl of Optimem™ (Gibco/BRL, Gaithersburg, MD). 10 µg of Lipofectamine™ (Gibco/BRL, Gaithersburg, MD) was added to a second 200 µl volume of Optimem™. The two solutions were mixed, incubated at room temperature for 30 minutes and adjusted to 600 µl with Optimem™. Cells were washed twice with phosphate buffered saline. The DNA-liposome complex was applied to cells. Cells were incubated for 6 hours at which time 2 ml of growth medium was added to cells. 16 hours later, the transfection mixture was removed and 2 ml fresh growth medium was added.

EXAMPLE 4

Luciferase Assay

Approximately 24 hours after transfection, various amounts of recombinant human leptin were added to cells. Cells were then incubated for 24 hours. Cell culture medium was removed and cells were washed promptly with 2 ml of ice-cold calcium and magnesium-

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free phosphate buffered saline. Cells were then prepared for assay with 150 μ l Promega reporter lysis reagent (Promega, Madison, WI) according to the manufacturer's protocol. 20 μ l of cell lysates was then assayed for luciferase activity using a Dynatech ML 3000 luminometer (Dynatech, Chantilly, VA) using cycle mode after initiating the reaction with 100 μ l of Promega luciferase assay reagent. The β -galactosidase activity in 50 μ l of each cell lysate was determined using a β -galactosidase assay (Promega, Madison, WI). Results were normalized by dividing the relative light units obtained for each sample by the β -galactosidase activity.

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WHAT IS CLAIMED IS:

1. A method for determining if a leptin receptor binding compound is present in a sample comprising:
5 contacting the sample with a cell which comprises a) nucleic acids comprising a promoter region, said promoter region comprising a promoter and at least one leptin response element; said promoter region operatively linked to a reporter gene; and b) nucleic acids encoding a leptin receptor; and
10 determining if transcription of the reporter gene occurs.
2. A method according to Claim 1 wherein the leptin response element is an IRF-1 derived gamma-interferon activation sequence.
15
3. A method according to Claim 2 wherein the IRF-1 derived gamma interferon activation sequence is
CTG ATT TCC CCG AAA TGA CG.
- 20 4. A method according to Claim 3 wherein the promoter region comprises a plurality of leptin response elements.
5. A method according to Claim 4 wherein the promoter region comprises at least three IRF-1 derived gamma-interferon activated sequences.
25
6. A method according to Claim 3 wherein the leptin receptor is a recombinant leptin receptor.
- 30 7. A method according to Claim 6 wherein the leptin receptor is human or rodent.

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8. A method according to Claim 7 wherein the nucleic acid comprising the leptin receptor comprises a sequence selected from the group of sequences consisting of those in Figures 3, 4 and 5.

5 9. A method according to Claim 3 wherein the transcription of the reporter gene occurs in proportion to the amount of leptin receptor binding compound present.

10 10. A nucleic acid comprising a promoter region, said promoter region comprising a promoter and at least one leptin response element; said promoter region operatively linked to a reporter gene.

15 11. A nucleic acid according to Claim 10 wherein the leptin response element is CTG ATT TCC CCG AAA TGA CG.

12. A nucleic acid according to Claim 11 wherein the promoter is a minimal promoter.

20 13. A nucleic acid according to Claim 12 wherein the reporter gene is luciferase.

25 14. A vector comprising nucleic acids comprising a promoter region, said promoter region comprising a promoter and at least one leptin response element, and said promoter region operatively linked to a reporter gene.

30 15. A set of vectors comprising
a) a first vector comprising a promoter region, said promoter region comprising a promoter and at least one leptin response element, said promoter region operatively linked to a reporter gene; and
b) a second vector comprising nucleic acids encoding a leptin receptor.

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16. A set of vectors according to Claim 15, wherein the second vector comprises a sequence selected from the group of sequences consisting of those in Figures 3, 4 and 5.

5 17. A cell which has been transfected with a) a vector comprising nucleic acids comprising a promoter region, said promoter region comprising a promoter and at least one leptin-response element, and said promoter region operatively linked to a reporter gene; and b) nucleic acids encoding a leptin receptor.

10 18. A method for quantifying the amount of a leptin-receptor binding compound present in a sample comprising:
contacting the sample with a cell which has been transfected with a) nucleic acids comprising a promoter region, said promoter
15 region comprising a promoter and at least one leptin response element; said promoter region operatively linked to a reporter gene; and b) nucleic acids encoding a leptin receptor; and
determining the amount of transcription of the reporter
gene that occurs.

20 19. A method of determining if a putative leptin response element is a leptin response element comprising:
providing a cell transfected with a construct comprising a
promoter region, said promoter region comprising a promoter and the
25 putative leptin response element, said promoter region operatively linked to a reporter gene, and said cell expressing leptin receptor; and
contacting said cell with leptin.

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20. A method for determining the leptin binding ability of a putative leptin receptor comprising:

- 5 providing a cell which expresses the putative leptin receptor, said cell also comprising nucleic acids comprising a promoter region, said promoter region comprising a promoter and at least one leptin response element, and wherein said promoter region is operatively linked to a reporter gene; and contacting the cell with leptin.

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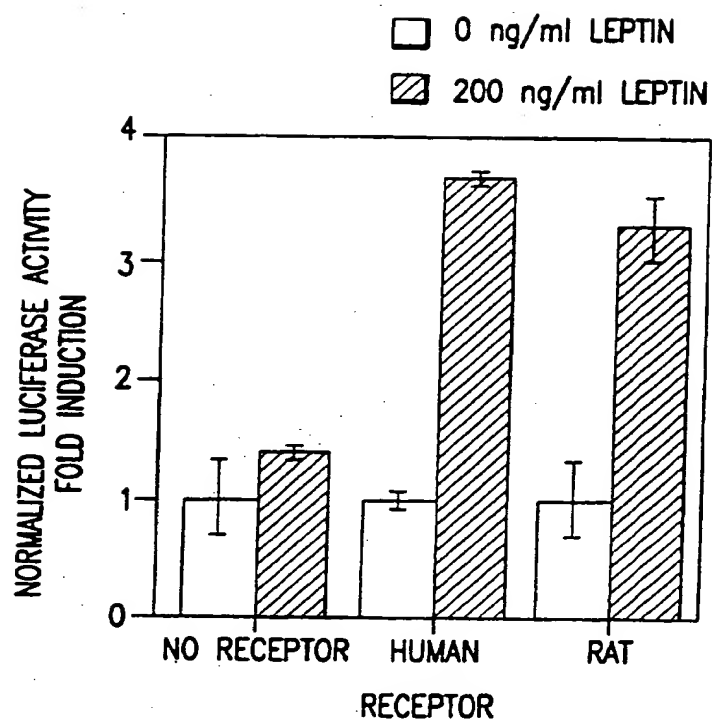


FIG.1

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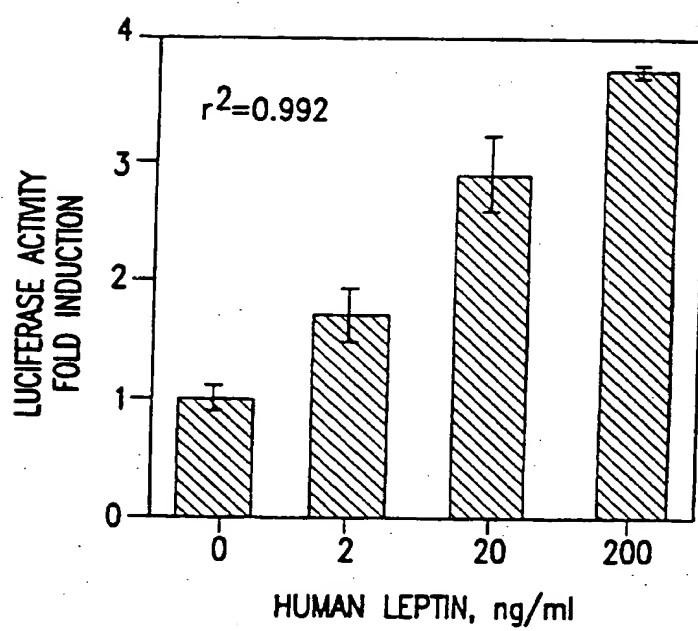


FIG.2

1 TGGGGCAATT GGGCTGACCT TTCTTATGCT GGGATGTGCC TTGGAGGACT
51 ATGGGTGTCT ATCTCTGAAG TAAGATGACG TGTCAGAAAT TCTATGTGGT
101 TTTGTTACAC TGGGAATTTT TGTATGTGAT AACTGCACTT AACCTGGCCT
151 ATCCAACCTC TCCCTGGAGA TTTAAGCTGT TTTGTGCGCC ACCGAGTACA
201 ACTGATGACT CCTTTCTCTC TCCTGCTGGA GTCCCAAACA ATACTTCGTC
251 TTTGAAGGGG GCTTCTGAAG CACTTGTTGA AGCTAAATTT AATTCAACTG
301 GTATCTACGT TTCTGAGTTA TCCAAAACCA TTTTCCACTG TTGCTTTGGG
351 AATGAGCAAG GTCAAACTG CTCCGCACTC ACAGGCAACA CTGAAGGGAA
401 GACGCTGGCT TCAGTGGTGA AGCCTTTAGT TTTCCGCCAA CTAGGTGTAA
451 ACTGGGACAT AGAGTGCTGG ATGAAAGGGG ACTTGACATT ATTCATCTGT
501 CATATGGAAC CATTACTTAA GAACCCCTTC AAGAATTATG ACTCTAAGGT
551 TCACCTTTTA TATGATCTGC CTGAAGTTAT AGATGATTTG CCTCTGCCCC
601 CACTGAAAGA CAGCTTTCAG ACTGTCCAGT GCAACTGCAG TGTTCCGGAA
651 TGCGAATGTC ATGTACCACT ACCCAGAGCC AAAGTCAACT ACGCTCTTCT
701 GATGTATTTA GAAATCACAT CTGCTGGTGT GAGTTTTTCA TCACCTCTAA
751 TGTCACTGCA GCCCATGCTT GTTGTGAAGC CCGATCCACC GCTGGGTTTG
801 CGTATGGAAG TCACAGATGA TGGTAATTTA AAGATTTTCA GGGACAGCCA
851 AACAAAAGCA CCATTTCCAC TTCAATATCA GGTGAAATAT TTAGAGAATT
901 CTACAATCGT AAGAGAGGCT GCTGAAATCG TCTCGGATAC ATCTCTGCTG
951 GTAGACAGCG TGCTTCTGG GTCTTCATAC GAGGTCCAGG TGAGGAGCAA
1001 GAGACTGGAT GGCTCAGGAG TCTGGAGTGA CTGGAGTTTA CCTCAACTCT
1051 TTACCACACA AGATGTCATG TATTTTCCAC CCAAATTCT GACGAGTGTT
1101 GGATCCAATG CTTCTTTTG CTGCATCTAC AAAAATGAGA ACCAGACTAT
1151 CTCCTCAAAA CAAATAGTTT GGTGGATGAA TCTAGCCGAG AAGATCCCCG
1201 AGACACAGTA CAACACTGTG AGTGACCACA TTAGCAAAGT CACTTTCTCC
1251 AACCTGAAAG CCACCAGACC TCGAGGGAAG TTTACCTATG ATGCAGTGTA
1301 CTGCTGCAAT GAGCAGGCAT GCCATCACCG CTACGCTGAA TTATATGTGA
1351 TCGATGTCAA TATCAATATA TCATGTGAAA CTGACGGGTA CTTAATAAAA
1401 ATGACTTGCA GATGGTCACC CAGCACAATC CAATCACTAG TGGGAAGCAC
1451 TGTGCAGTTG AGGTATCACA GGCGCAGCCT GTACTGTCCC GATAATCCAT
1501 CTATTCGTCC TACATCAGAG CTCAAAACT GCGTCTTACA GACAGATGGC
1551 TTTTATGAAT GTGTTTTCCA GCCAATCTTT CTATTATCTG GCTATACAAT
1601 GTGGATCAGG ATCAACCATT CTTTAGGTTT ACTTGACTCT CCACCAACGT
1651 GTGTCCTTCC TGAATCCGTA GTAAAACCAC TACCTCCATC TAATGTAAAA
1701 GCAGAGATTA CTATAAACAC TGGATTATTG AAAGTATCTT GGGAAAAGCC
1751 AGTCTTTCCA GAGAATAACC TTCAGTTCCA GATTCGATAT GGCTTAAATG
1801 GAAAAGAAAT ACAATGGAAG ACACACGAGG TATTCGATGC AAAATCAAAA
1851 TCGGCCAGCC TGCCAGTGTC AGATCTCTGT GCGGTCTATG TGGTACAGGT
1901 TCGCTGCCGG CGGTTGGATG GACTAGGGTA TTGGAGTAAT TGGAGCAGTC
1951 CAGCCTACAC TCTTGTCATG GATGTAAAAG TTCCTATGAG AGGGCCTGAA
2001 TTCTGGAGAA TAATGGATGG GGATATTACT AAAAAGGAGA GAAATGTCAC

FIG.3A

2051 CTTGCTTTGG AAGCCACTGA TGAAAAATGA CTCACTGTGT AGTGTGAGGA
2101 GGTATGTGGT GAAGCATCGT ACTGCCCACA ATGGGACATG GTCACAAGAT
2151 GTGGGAAATC AGACCAATCT CACTTTCCTG TGGGCAGAAT CAGCACACAC
2201 TGTTACAGTT CTGGCCATCA ATTCCATCGG TGCCTCCCTT GTGAATTTTA
2251 ACCTTACGTT CTCATGGCCC ATGAGTAAAG TGAATGCTGT GCAGTCACTC
2301 AGTGCTTATC CCCTGAGCAG CAGCTGCGTC ATCCTTTCCT GGACACTGTC
2351 ACCTAATGAT TATAGTCTGT TATATCTGGT TATTGAATGG AAGAACCTTA
2401 ATGATGATGA TGGAAATGAAG TGGCTTAGAA TCCCTTCGAA TGTTAACAAG
2451 TATTATATCC ATGATAATTT TATTCCTATC GAGAAATATC AGTTTAGTCT
2501 TTACCCAGTA TTTATGGAAG GAGTTGGAAG ACCAAAGATA ATTAATGGTT
2551 TCACCAAAGA TGATATCGCC AAACAGCAAA ATGATGCAGG GCTGTATGTC
2601 ATTGTACCGA TAATTATTTT CTCTTGTCGTC CTGCTGCTCG GAACACTGTT
2651 AATTTACACAC CAGAGAATGA AAAAGTTGTT TTGGGACGAT GTTCCAAACC
2701 CCAAGAATTG TTCCTGGGCA CAAGGACTTA ATTTCCAAAA GCCTGAAACA
2751 TTTGAGCATC TTTTACCAA GCATGCAGAA TCAGTGATAT TTGGTCCTCT
2801 TCTTCTGGAG CCTGAACCAG TTTCAGAAGA AATCAGTGTC GATACAGCTT
2851 GGAAAAATAA AGATGAGATG GTACCAGCAG CTATGGTCTC ACTTCTTTTG
2901 ACCACTCCAG ATTCCACAAG GGGTTCTATT TGTATCAGTG ACCAGTGTA
2951 CAGTGCTAAC TTCTCTGGGG CTCAGAGCAC CCAGGGAACC TGTGAGGATG
3001 AGTGTCAGAG TCAACCCTCA GTTAAATATG CAACGCTGGT CAGCAACGTG
3051 AAAACAGTGG AAACATGATGA AGAGCAAGGG GCTATACATA GTTCTGTCAG
3101 CCAGTGCATC GCCAGGAAAC ATTCCCACT GAGACAGTCT TTTCTAGCA
3151 ACTCCTGGGA GATAGAGGCC CAGGCATTTT TCCTTTTATC AGATCATCCA
3201 CCCAATGTGA TTTCACCACA ACTTTCATTC TCAGGGTTGG ATGAGCTTTT
3251 GGAACTGGAG GGAAATTTTC CTGAAGAAAA TCACGGGGAA AAATCTGTGT
3301 ATTATCTAGG AGTCTCCTCA GGAAACAAAA GAGAGAATGA TATGCTTTTG
3351 ACTGATGAGG CAGGGGTATT GTGCCCATTC CCAGCTCACT GTCTGTTTCA
3401 TGACATCAGA ATCCTCCAGG AGAGTTGTTT ACACCTTTGTA GAAAAATAAT
3451 TGAATTTAGG GACCTCTGGT AAGAACTTTG TACCTTACAT GCCCCAGTTT
3501 CAATCCTGTT CCACTCACAG TCATAAGATA ATAGAAAATA AGATGTGTGA
3551 CTAACTGTG TAATCTTGTC CAAAACTTC CAGGTTCCAT TCCAGTAGAG
3601 TGTGTCATGT ATAATATGTT CTTTTATAGT TGTGGGTGGG AGAGAAAGCC

FIG.3B

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141 CTTATGCTG
151 GGATGTGCCT TAGAGGATTA TGGGTGTACT TCTCTGAAGT AAGATGATTT
201 GTCAAAAATT CTGTGTGGTT TTGTTACATT GGGAATTTAT TTATGTGATA
251 ACTGCGTTTA ACTTGTGATA TCCAATTACT CCTTGGAGAT TTAAGTTGTC
301 TTGCATGCCA CCAAATTCOA CCTATGACTA CTTCTTTTG CCTGCTGGAC
351 TCTCAAAGAA TACTTCAAAT TCGAATGGAC ATTATGAGAC AGCTGTTGAA
401 CCTAAGTTTA ATTCAAGTGG TACTCACTTT TCTAACTTAT CCAAAACAAC
451 TTTCCACTGT TGCTTTCGGA GTGAGCAAGA TAGAACTGC TCCTTATGTG
501 CAGACAACAT TGAAGGAAAG ACATTTGTTT CAACAGTAAA TTCTTTAGTT
551 TTTCAACAAA TAGATGCAAA CTGGAACATA CAGTGCTGGC TAAAAGGAGA
601 CTTAAAATTA TTCATCTGTT ATGTGGAGTC ATTATTTAAG AATCTATTCA
651 GGAATTATAA CTATAAGGTC CATCTTTTAT ATGTTCTGCC TGAAGTGTTA
701 GAAGATTCAC CTCTGGTTCC CCAAAAAGGC AGTTTTCAGA TGGTTCACTG
751 CAATTGCAGT GTTCATGAAT GTTGTGAATG TCTTGTGCCT GTGCCAACAG
801 CCAAACCTCA CGACACTCTC CTTATGTGTT TGAATAACAC ATCTGGTGGA
851 GTAATTTTCC AGTCACCTCT AATGTCAGTT CAGCCCATAA ATATGGTGAA
901 GCCTGATCCA CCATTAGGTT TGCATATGGA AATCACAGAT GATGGTAATT
951 TAAAGATTTT TTGGTCCAGC CCACCATTGG TACCATTTC ACTTCAATAT
1001 CAAGTGAAAT ATTCAGAGAA TTCTACAACA GTTATCAGAG AAGCTGACAA
1051 GATTGTCTCA GCTACATCCC TGCTAGTAGA CAGTATACTT CCTGGGTCTT
1101 CGTATGAGGT TCAGGTGAGG GGCAAGAGAC TGGATGGCCC AGGAATCTGG
1151 AGTGACTGGA GTACTCCTCG TGTCTTTACC ACACAAGATG TCATATACTT
1201 TCCACCTAAA ATTCTGACAA GTGTTGGGTC TAATGTTTCT TTCACTGCA
1251 TCTATAAGAA GGAAAACAAG ATTGTTCCCT CAAAAGAGAT TGTTTGGTGG
1301 ATGAATTTAG CTGAGAAAAT TCCTCAAAGC CAGTATGATG TTGTGAGTGA
1351 TCATGTTAGC AAAGTTACTT TTTTCAATCT GAATGAAACC AAACCTCGAG
1401 GAAAGTTTAC CTATGATGCA GTGTACTGCT GCAATGAACA TGAATGCCAT
1451 CATCGCTATG CTGAATTATA TGTGATTGAT GTCAATATCA ATATCTCATG
1501 TGAACTGAT GGGTACTTAA CTAATATGAC TTGCAGATGG TCAACCAGTA
1551 CAATCCAGTC ACTTGCGGAA AGCACTTTGC AATTGAGGTA TCATAGGAGC
1601 AGCCTTTACT GTTCTGATAT TCCATCTATT CATCCCATAT CTGAGCCCAA
1651 AGATTGCTAT TTGCAGAGTG ATGGTTTTTA TGAATGCATT TTCCAGCCAA
1701 TCTTCCTATT ATCTGGCTAC ACAATGTGGA TTAGGATCAA TCACTCTCTA
1751 GGTTCACTTG ACTCTCCACC AACATGTGTC CTTCTGATT CTGTGGTGAA
1801 GCCACTGCCT CCATCCAGTG TGAAAGCAGA AATTACTATA AACATTGGAT
1851 TATTGAAAAT ATCTTGGGAA AAGCCAGTCT TTCCAGAGAA TAACCTTCAA
1901 TTCCAGATTC GCTATGGTTT AAGTGGAAAA GAAGTACAAT GGAAGATGTA
1951 TGAGGTTTAT GATGCAAAAT CAAAATCTGT CAGTCTCCA GTTCCAGACT
2001 TGTGTGCAGT CTATGCTGTT CAGGTGCGCT GTAAGAGGCT AGATGGACTG
2051 GGATATTGGA GTAATTGGAG CAATCCAGCC TACACAGTTG TCATGGATAT
2101 AAAAGTTCCT ATGAGAGGAC CTGAATTTTG GAGAATAATT AATGGAGATA

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FIG.4A

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2151 CTATGAAAAA GGAGAAAAAT GTCACCTTAC TTTGGAAGCC CCTGATGAAA
2201 AATGACTCAT TGTGCAGTGT TCAGAGATAT GTGATAAACC ATCATACTTC
2251 CTGCAATGGA ACATGGTCAG AAGATGTGGG AAATCACACG AAATTCACCT
2301 TCCTGTGGAC AGAGCAAGCA CATACTGTTA CGGTTCTGGC CATCAATTCA
2351 ATTGGTGCTT CTGTTGCAAA TTTTAATTTA ACCTTTTCAT GGCCTATGAG
2401 CAAAGTAAAT ATCGTGCACT CACTCAGTGC TTATCCTTTA AACAGCAGTT
2451 GTGTGATTGT TTCCTGGATA CTATCACCCA GTGATTACAA GCTAATGTAT
2501 TTTATTATTG AGTGGAAAAA TCTTAATGAA GATGGTGAAA TAAAATGGCT
2551 TAGAATCTCT TCATCTGTTA AGAAGTATTA TATCCATGAT CATTTTATCC
2601 CCATTGAGAA GTACCAAGTC AGTCTTTACC CAATATTTAT GGAAGGAGTG
2651 GGAAAACCAA AGATAATTAA TAGTTTCACT CAAGATGATA TTGAAAAACA
2701 CCAGAGTGAT GCAGGTTTAT ATGTAATTGT GCCAGTAATT ATTCCTCTT
2751 CCATCTTATT GCTTGGAACA TTATTAATAT CACACCAAAG AATGAAAAAG
2801 CTATTTTGGG AAGATGTTCC GAACCCCAAG AATTGTTCTT GGGCACAAGG
2851 ACTTAATTTT CAGAAGCCAG AAACGTTTGA GCATCTTTTT ATCAAGCATA
2901 CAGCATCAGT GACATGTGGT CCTCTTCTTT TGGAGCCTGA AACAATTTCA
2951 GAAGATATCA GTGTTGATAC ATCATGGAAA AATAAAGATG AGATGATGCC
3001 AACAACGTGT GTCTCTCTAC TTTCAACAAC AGATCTTGAA AAGGGTCTG
3051 TTTGTATTAG TGACCAGTTC AACAGTGTTA ACTTCTCTGA GGCTGAGGGT
3101 ACTGAGGTAA CCTATGAGGC CGAAAGCCAG AGACAACCCT TTGTAAATA
3151 CGCCACGCTG ATCAGCAACT CTAAACCAAG TGAACTGGT GAAGAACAAG
3201 GGCTTATAAA TAGTTCAGTC ACCAAGTGCT TCTCTAGCAA AAATTCTCCG
3251 TTGAAGGATT CTTTCTCTAA TAGCTCATGG GAGATAGAGG CCCAGGCATT
3301 TTTTATATTA TCAGATCAGC ATCCCAACAT AATTCACCA CACCTCACAT
3351 TCTCAGAAGG ATTGGATGAA CTTTTGAAAT TGGAGGGAAA TTTCCCTGAA
3401 GAAAATAATG ATAAAAAGTC TATCTATTAT TTAGGGGTCA CCTCAATCAA
3451 AAAGAGAGAG AGTGGTGTGC TTTTGACTGA CAAGTCAAGG GTATCGTGCC
3501 CATTCCCAGC CCCCTGTTTA TTCACGGACA TCAGAGTTCT CCAGGACAGT
3551 TGCTCACACT TTGTAGAAAA TAATATCAAC TTAGGAACTT CTAGTAAGAA
3601 GACTTTTGCA TCTTACATGC CTCAATTCCA AACTTGTCT ACTCAGACTC
3651 ATAAGATCAT GGAAAACAAG ATGTGTGACC TAACTGTGTA ATTTCACTGA
3701 AGAAACCTTC AGATTTGTGT TATAATGGGT AATATAAAGT GTAATAGATT
3751 ATAGTTGTGG GTGGGAGAGA

FIG.4B

1 TGGGGCAATT GGGCTGACCT TTCTTATGCT GGGATGTGCC TTGGAGGACT
51 ATGGGTGTCT ATCTCTGAAG TAAGATGACG TGTCAGAAAT TCTATGTGGT
101 TTTGTTACAC TGGGAATTC TGTATGTGAT AACTGCACTT AACCTGGCCT
151 ATCCAACCTC TCCCTGGAGA TTTAAGCTGT TTTGTGCGCC ACCGAGTACA
201 ACTGATGACT CCTTTCTCTC TCCTGCTGGA GTCCCAAACA ATACTTCGTC
251 TTTGAAGGGG GCTTCTGAAG CACTTGTTGA AGCTAAATTT AATTCAACTG
301 GTATCTACGT TTCTGAGTTA TCCAAAACCA TTTTCCACTG TTGCTTTGGG
351 AATGAGCAAG GTCAAAACTG CTCCGCACTC ACAGGCAACA CTGAAGGGAA
401 GACGCTGGCT TCAGTGGTGA AGCCTTTAGT TTTCCGCCAA CTAGGTGTAA
451 ACTGGGACAT AGAGTGTGG ATGAAAGGGG ACTTGACATT ATTCATCTGT
501 CATATGGAAC CATTACTTAA GAACCCCTTC AAGAATTATG ACTCTAAGGT
551 TCACCTTTTA TATGATCTGC CTGAAGTTAT AGATGATTTG CCTCTGCCCC
601 CACTGAAAGA CAGCTTTCAG ACTGTCCAGT GCAACTGCAG TGTTCCGGAA
651 TGCGAATGTC ATGTACCACT ACCCAGAGCC AAAGTCAACT ACGCTCTTCT
701 GATGTATTTA GAAATCACAT CTGCTGGTGT GAGTTTTTCTG TCACCTCTAA
751 TGTCAGTGCA GCCCATGCTT GTTGTGAAGC CTGGAGTTTA CCTCAACTCT
801 CGTATGGAAG TCACAGATGA TGGTAATTTA AAGATTTTCTG GGGACAGCCA
851 AACAAAAGCA CCATTTCAC TTCAATATCC GGTGAAATAT TTAGAGAATT
901 CTACAATCGT AAGAGAGGCT GCTGAAATCG TCTCGGATAC ATCTCTGCTG
951 GTAGACAGCG TGCTTCTGG CTGTTTCATAC GAGGTCCAGG TGAGGAGCAA
1001 GAGACTGGAT GGCTCAGGAG TCTGGAGTGA CTGGAGTTTA CCTCAACTCT
1051 TTACCACACA AGATGTCATG TATTTTCCAC CAAAATTCT GACGAGTGT
1101 GGATCCAATG CTTCTTTTGT CTGCATCTAC AAAAATGAGA ACCAGACTAT
1151 CTCCTCAAAA CAAATAGTTT GGTGGATGAA TCTAGCCGAG AAGATCCCCG
1201 AGACACAGTA CAACACTGTG AGTGACCACA TTAGCAAAGT CACTTTCTCC
1251 AACCTGAAAG CCACCAGACC TCGAGGGAAG TTTACCTATG ATGCAGTGTA
1301 CTGCTGCAAT GAGCAGGCAT GCCATCACCG CTACGCTGAA TTATATGTGA
1351 TCGATGTCAA TATCAATATA TCATGTGAAA CTGACGGGTA CTAACTAAA
1401 ATGACTTGCA GATGGTCACC CAGCACAATC CAATCACTAG TGGGAAGCAC
1451 TGTGCAGTTG AGGTATCACA GGCGCAGCCT GTACTGTCCC GATAATCCAT
1501 CTATTCGTCC TACATCAGAG CTCAAAACT GCGTCTTACA GACAGATGGC
1551 TTTTATGAAT GTGTTTCCA GCCAATCTTT CTATTATCTG GCTATACAAT
1601 GTGGATCAGG ATCAACCATT CTTTAGGTTT ACTTGACTCT CCACCAACGT
1651 GTGTCCCTCC TGAATCCGTA GTAAAACCAC TACCTCCATC TAATGTAAAA
1701 GCAGAGATTA CTATAAACAC TGGATTATTG AAAGTATCTT GGGAAAAGCC
1751 AGTCTTTCCA GAGAATAACC TTCAGTTCCA GATTTCGATAT GGCTTAAATG
1801 GAAAAGAAAT ACAATGGAAG ACACACGAGG TATTCGATGC AAAATCAAAA
1851 TCGGCCAGCC TGCCAGTGTG AGATCTCTGT GCGGTCTATG TGGTACAGGT
1901 TCGCTGCCGG CGGTTGGATG GACTAGGGTA TTGGAGTAAT TGGAGCAGTC
1951 CAGCCTACAC TCTTGTCTAG GATGTAAAAG TTCCTATGAG AGGGCCTGAA
2001 TTCTGGAGAA TAATGGATGG GGATATTACT AAAAAGGAGA GAAATGTCAC
2051 CTTGCTTTGG AAGCCACTGA TGAAAAATGA CTCAGTGTGT AGTGTGAGGA

FIG. 5A

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2101 GGTATGTGGT GAAGCATCGT ACTGCCCACA ATGGGACATG GTCACAAGAT
2151 GTGGGAAATC AGACCAATCT CACTTTCCTG TGGGCAGAAT CAGCACACAC
2201 TGTTACAGTT CTGGCCATCA ATTCCATCGG TGCCTCCCTT GTGAATTTTA
2251 ACCTTACGTT CTCATGGCCC ATGAGTAAAG TGAATGCTGT GCAGTCACTC
2301 AGTGCTTATC CCCTGAGCAG CAGCTGCGTC ATCCTTTCCT GGACACTGTC
2351 ACCTAATGAT TATAGTCTGT TATATCTGGT TATTGAATGG AAGAACCTTA
2401 ATGATGATGA TGGAAATGAAG TGGCTTAGAA TCCCTTCGAA TGTTAAACAAG
2451 TATTATATCC ATGATAATTT TATTCCTATC GAGAAATATC AGTTTAGTCT
2501 TTACCCAGTA TTTATGGAAG GAGTTGGAAA ACCAAAGATA ATTAATGGTT
2551 TCACCAAAGA TGATATCGCC AAACAGCAAA ATGATGCAGG GCTGTATGTC
2601 ATTGTACCGA TAATTATTTT CTCTTGTGTC CTGCTGCTCG GAACACTGTT
2651 AATTTACAC CAGAGAATGA AAAAGTTGTT TTGGGACGAT GTTCCAAACC
2701 CCAAGAATTG TTCCTGGGCA CAAGGACTTA ATTTCCAAAA GCCTGAAACA
2751 TTTGAGCATC TTTTTACCAA GCATGCAGAA TCAGTGATAT TTGGTCCTCT
2801 TCTTCTGGAG CCTGAACCAG TTTCAGAAGA AATCAGTGTC GATACAGCTT
2851 GGAAAAATAA AGATGAGATG GTACCAGCAG CTATGGTCTC ACTTCTTTTG
2901 ACCACTCCAG ATTCCACAAG GGGTTCTATT TGTATCAGTG ACCAGTGTA
2951 CAGTGCTAAC TTCTCTGGGG CTCAGAGCAC CCAGGGAACC TGTGAGGATG
3001 AGTGTCAGAG TCAACCCTCA GTTAAATATG CAACGCTGGT CAGCAACGTG
3051 AAAACAGTGG AAACCTGATGA AGAGCAAGGG GCTATACATA GTTCTGTCAG
3101 CCACTGCATC GCCAGGAAAC ATTCCCACT GAGACAGTCT TTTTCTAGCA
3151 ACTCCTGGGA GATAGAGGCC CAGGCATTTT TCCTTTTATC AGATCATCCA
3201 CCAATGTGA TTTACCACA ACTTTCATTC TCAGGGTTGG ATGAGCTTTT
3251 GGAACCTGGAG GGAAATTTTC CTGAAGAAAA TCACGGGGAA AAATCTGTGT
3301 ATTATCTAGG AGTCTCCTCA GGAAACAAAA GAGAGAATGA TATGCTTTTG
3351 ACTGATGAGG CAGGGGTATT GTGCCCATTC CCAGCTCACT GTCTGTTTCA
3401 TGACATCAGA ATCCTCCAGG AGAGTTGTTT ACACTTTGTA GAAAAAATT
3451 TGAATTTAGG GACCTCTGGT AAGAACTTTG TACCTTACAT GCCCCAGTTT
3501 CAATCCTGTT CCACTCACAG TCATAAGATA ATAGAAAATA AGATGTGTGA
3551 CTTAACTGTG TAATCTTGTC CAAAACTTC CAGGTTCCAT TCCAGTAGAG
3601 TGTGTCATGT ATAATATGTT CTTTTATAGT TGTGGGTGGG AGAGAAAGCC

FIG.5B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/06505

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : G01N 33/53; C12N 1/20, 15/00, 15/12, 15/19

US CL : Please See Extra Sheet

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/ 7.1, 7.2, 7.21, 7.32, 7.8, 320.1, 252.3, 325, 335, 336; 536/24.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS; ONLINE, DIALOG, MEDLINE; search terms ob/leptin receptor and promoter, binding assay/compounds

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TARTAGLIA et al. Identification and expression cloning of a leptin receptor, OB-R. Cell. 29 December 1995, Vol. 83, pages 1263-1271, see entire document.	1-20
X	LEE et al. Abnormal splicing of the leptin receptor in <i>diabetic</i> mice. Nature. 15 February 1996, Vol. 379, pages 332-335, see entire document.	1-20
X	CHEN et al. Evidence that the diabetes gene encodes the leptin receptor: Identification of a mutation in the leptin receptor gene in <i>db/db</i> mice. Cell. 09 February 1996, Vol. 84, pages 491-495, see entire document.	1-20
X, E	US, 5,643,748 A (SNODGRASS ET AL) 01 July 1997 (01.07.97), see entire document.	1-20

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

02 SEPTEMBER 1997

Date of mailing of the international search report

30 SEP 1997

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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/06505

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category ^a	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	CIOFFI et al. Novel B219/OB receptor isoforms: Possible role of leptin in hematopoiesis and reproduction. <i>Nature Medicine</i> . May 1996, Vol. 2, No. 5, pages 585-589, see entire document.	1-20
X, P	WANG et al. A novel leptin receptor isoform in rat. <i>FEBS Letters</i> . August 1996, Vol. 392, pages 87-90, see entire document.	1-20
X, P	IIDA et al. Phenotype-linked amino acid alteration in leptin receptor cDNA from Zucker fatty (<i>fa/fa</i>) rat. <i>Biochem. Biophys. Res. Comm.</i> May 1996, Vol. 222, pages 19-26, see entire document.	1-20
X	PINE et al. Tyrosine phosphorylated p91 binds to a single element in the ISGF2/IF-1 promoter to mediate induction by IFN α and IFN γ , and is likely to autoregulate the p91 gene. <i>The EMBO J.</i> 1994, Vol. 13, No. 1, pages 158-167, see entire document.	1-20
X	WO 92/02639 A1 (THE SALK INSTITUTE BIOTECHNOLOGY/INDUSTRY ASSOCIATES, INC.) 20 February 1992 (20.02.92), see the entire document, especially the claims.	1-20
X	HODGSON, J. Receptor screening and the search for new pharmaceuticals. <i>Bio/Technology</i> . September 1992, Vol. 10, pages 973-977, see entire document.	1-20
X, P	ROSENBLUM et al. Functional STAT 1 and 3 signaling by the leptin receptor (OB-R); reduced expression of the rat <i>fatty</i> leptin receptor in transfected cells. <i>Endocrinology</i> . November 1996, Vol. 137, No. 11, pages 5178-5181, see entire document.	1-20
X, P	GHILARDI et al. Defective STAT signaling by the leptin receptor in <i>diabetic</i> mice. <i>Proc. Natl. Acad. Sci. USA</i> . June 1996, Vol. 93, pages 6231-6235, see entire document.	1-20
X, P	BAUMANN et al. The full-length leptin receptor has signalling capabilities of interleukin 6-type cytokine receptors. <i>Proc. Natl. Acad. Sci. USA</i> . August 1996, Vol. 93, pages 8374-8378, see entire document.	1-20
X, P	WO 96/38586 A1 (SMITHKLINE BEECHAM PLC) 05 December 1996 (05.12.96), see entire document.	1-20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/06505

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/7.1, 7.2, 7.21, 7.32, 7.8, 320.1, 252.3, 325, 335, 336; 536/24.1